

BIOMAGNETISM AND FERRITIN

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I. OBJECTIVES

The first objectives have been to:

- (a) determine the influence, if any, of the magnetic field on the development of frogs' eggs and frog embryos to the hatching stage and beyond.
- (b) test the hypothesis that such an influence, if it occurs, is due to the mechanical forces of the magnetic field on ferritin in the frogs' eggs before Shumway Stage 25.

II. INITIAL WORK

The initial work consists of establishing a number of procedures:

- (a) the consistent and standardized development of frog embryos from fertilization through hatching.
- (b) the histological and cytological examination of these early embryos for the presence and distribution of ferritin using both light and electron microscopy, as well as a possible initial biochemical localization procedure.
- (c) magnetic field exposures under controlled conditions.

In all these categories of procedures we have made considerable progress but are still some distance from accomplishing all of our objectives.

Fertilization and Growth Procedures

- (a) Considerable variability occurs in the percentage of eggs which develop through hatching when one compares the results from one batch with another; successful hatching of more than 50% of all eggs was only obtained three times in 12 runs as indicated in Table 1.
- (b) The confined space which will be available in the magnet presents additional survival problems which remain to be worked out. First indications in various small dishes and in the proposed chamber for the magnet are that these conditions prevented successful hatching and therefore may require additional water circulation, different materials, or different concentrations of ions in the water.

- (c) Because of the seasonal fertility cycle of the Rana species no more Rana pipiens eggs will become available until December at best. Therefore, we have switched to Rana catesbeiana, the American bullfrog, for the summer. We do not expect these to react differently to magnetic field exposures. The size of the eggs and their rate of development through the stages of interest to us are very similar to Rana pipiens. In any case, we have no choice but to change the species of frog in order to continue our work the year around.

Histology, Cytology and Biochemical Assays

We have successfully worked out the following methods for obtaining sections:

- (a) Light microscopy: The developing eggs and embryos are taken at their desired stage and fixed, embedded, and sectioned as follows.
1. Fixation - in Bouin-Dioxane (half and half) fixative for 2 hours
 2. Dehydration - in Dioxane for 1 hour
 3. Embedding - in a low melting-point paraffin by first heating in a paraffin-dioxane-xylene mesh.
 4. Sectioning - at 10 microns

Embryos from every Shumway Stage 1 through 25 have been taken through step #3 of the above procedure, all obtained from one fertilization. Some of these have been sectioned and the procedure for doing so has been shown to be acceptable. Typical examples are the Shumway stages 1, 4, and 11, shown in Figures 2-4 respectively. The staining of these sections was as follows: Figure 2 was stained with hemotoxylin and eosin, Figure 3 and 4 with the Prussian blue reaction. Figure 4 shows some cells at the surface layers colored blue (see Figure 5) showing up dark in the black and white print. Since the Prussian blue reaction is specific for iron, one would suspect the presence of iron in these cells. The diffuse distribution of color in these cells, however, would indicate that this is only an artifact due to some kind of dye diffusion into these outermost layers. The embryo in Figure 2 showed no positive response whatever to the Prussian blue reaction. Up to this point we have concentrated on being able to cut good, usable sections and the above examples

are only the very first tries to develop some of the methods for staining of ferritin. This work is one of the next objectives on our program.

(b) Electron microscopy: Electron microscope sections were prepared by the following procedure using a different fixation than for light microscopy.

1. Fixed in 4% gluteraldehyde in s-collidine buffer, pH = 7.2, for 2 hours
2. Post-fixed in osmium tetroxide in s-collidine buffer for 3 hours
3. Dehydrated in ethanol (cold)
4. Embedded in Epon 812

In the electron micrographs the crystals of ferritin should be visible this way. To check our methods we have looked in the gut of some tadpoles where one might expect to find ferritin. Initial electromicrographs of tadpole liver indicate that we are making good progress in making suitable sections; however, particular tadpoles which we examined showed no ferritin. Probably it will be necessary to feed them an iron-rich diet for it to show up under these circumstances. (see Figures 6-10)

(c) Biochemical method: This work has led us to a further very promising approach towards an initial localization of the ferritin. There is some question as to how well ferritin can be stained, how much change the fixing and embedding procedures cause in the chemical composition of the cell. In addition, an adequate sample consisting of 10 microns thick sections from an object 2 millimeters thick is difficult to obtain with a reasonable number of sections. It has therefore been decided to go one step further towards a fundamental approach and localize ferritin by biochemical assays of e. g. 50 u thick serial cryo-sections of the eggs. Mazur and coworkers have recently worked out enzymatic methods of ferritin assay and we will give these a try before proceeding further with the microscopic methods.

Magnetic Field Exposure

For exposing frogs' eggs to a steady gradient magnetic field during the time that the ferritin is present in the eggs a large permanent magnet with pole pieces shaped to provide a high product of gradient and field strength was designed with the assistance of the General Electric Company and has just been delivered. At the same time the

initial design for a holder for the eggs was completed and fabricated. The objectives of the equipment design were to be able to expose the eggs at constant temperature conditions and to have exact duplicate holders at the same temperature, fluid flow rate, and concentration available to keep the controls and to be able to expose a reasonable number of eggs simultaneously to be able to draw some statistical conclusions. The results of these efforts will not become available until the next reporting period.

Temperature Control and Recording

A system was designed, ordered and delivered which, using 0.1°C precision thermistors, will be able to measure and record the temperature sequentially and continually at up to ten locations to this accuracy.

III. PLANS

It is expected that working out the procedures described above will be completed in the next few months so that we can then concentrate on getting results. The time schedule will depend somewhat on the supply of fertile eggs. The determination of the ferritin distribution is fortunately independent of these fertility vagaries and we will proceed with it systematically.

A check will be made on the results of P. C. Minnaar* on the development of Rhodeus Ocellatus eggs in magnetic fields and their relevance to this work.

* 2nd International Biomagnetism Symposium, Chicago, 1966

TABLE 1*

<u>Date of Fertilization</u>	<u>Overall Percentage of Eggs Hatching</u>
February 8	8 %
February 11	64 %
February 15	42 %
February 18	60 %
February 23	16 %
February 25	20 %
March 1	8 %
March 4	16 %
March 8	40 %
March 11	0 %
March 15**	72 %
March 18	40 %

* It should be noted that several different environments were used with eggs from each date of fertilization. This partially accounts for the varieties in percentages of hatching eggs from one trial to the next.

** Fixed preparation from all stages prepared.

FIGURE CAPTIONS

- Figure 1 10 micron section of frogs' egg stained with hematoxylin and eosin. Method of preparation is discussed in text. Mag = 66X
- Figure 2 10 micron section of frog embryo, stage 4 (4 cells). Only 3 of cells are visible because of plane of section. Fragmenting is common with delicate young embryos. Stained with Prussian blue (no reaction). Mag = 66X.
- Figure 3 10 micron section of frog embryo at stage 11 (late yolk plug). Stained with Prussian blue. Mag = 66X.
- Figure 4 Same section as Figure 3. Mag = 206X.
- Figure 5 500-1000A⁰ section of tadpole liver showing bile canaliculi, melamin, and fat droplets but no ferritin crystals. Mag = 4500X.
- Figure 6 Same thickness, but higher magnification. Mag = 9250X.
- Figure 7 Mag = 14,750X
- Figure 8 Mag = 18,750X
- Figure 9 Mag = 24,750X



Figure 1(1)



Figure 1(2)



Figure 1(3)

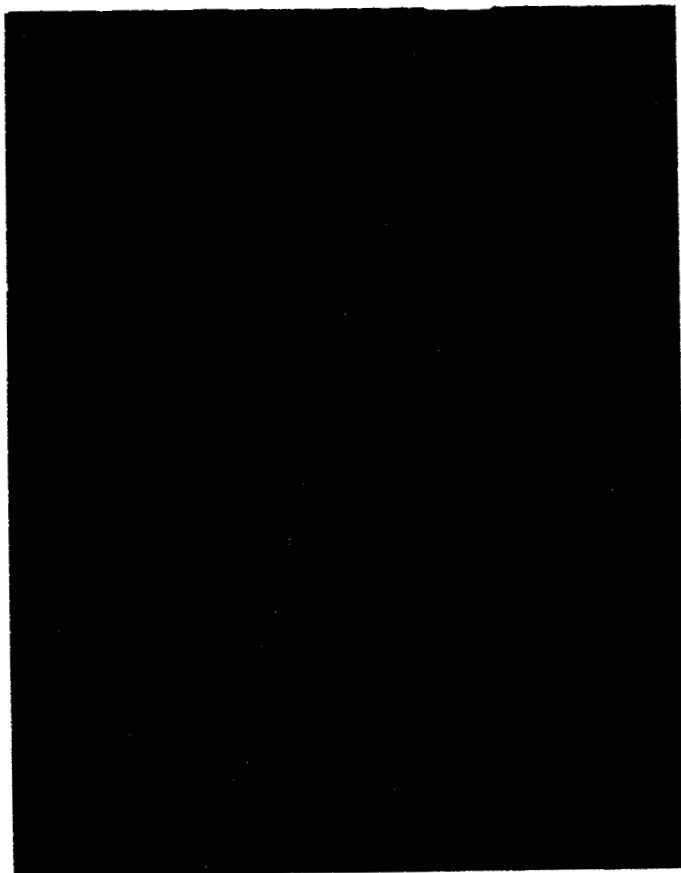


Figure 2(1)

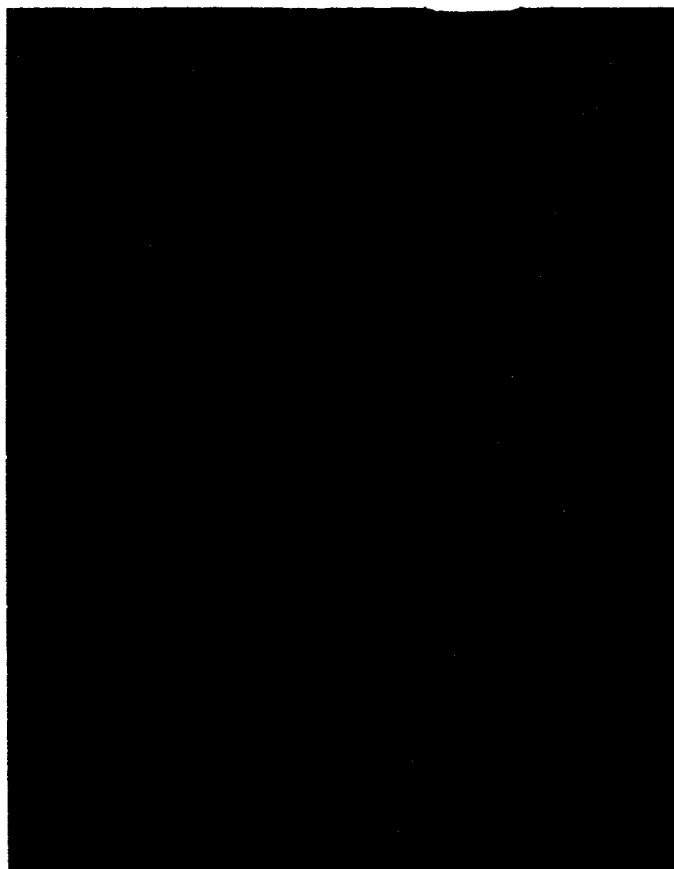


Figure 2(2)



Figure 2(3)

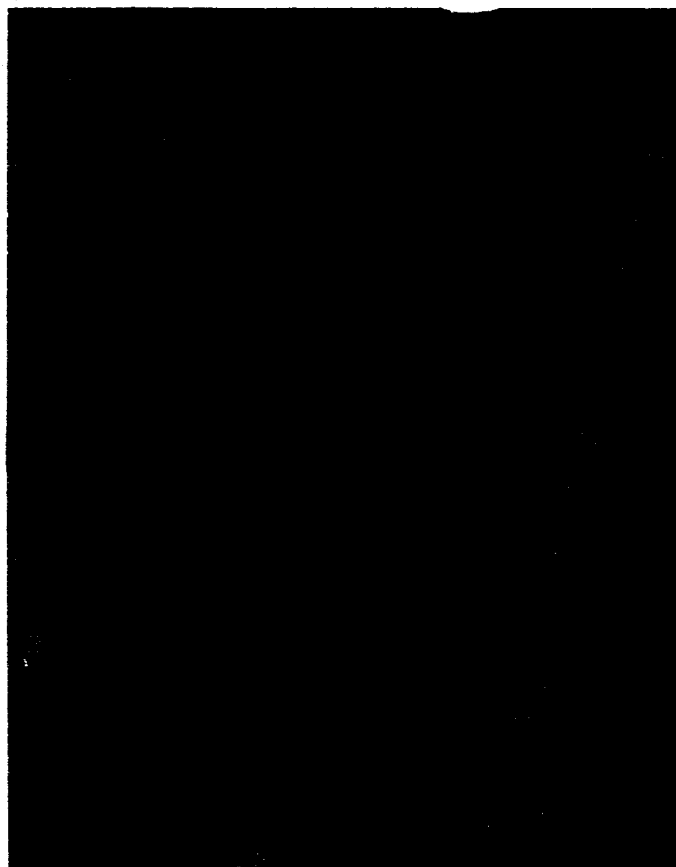


Figure 3(1)

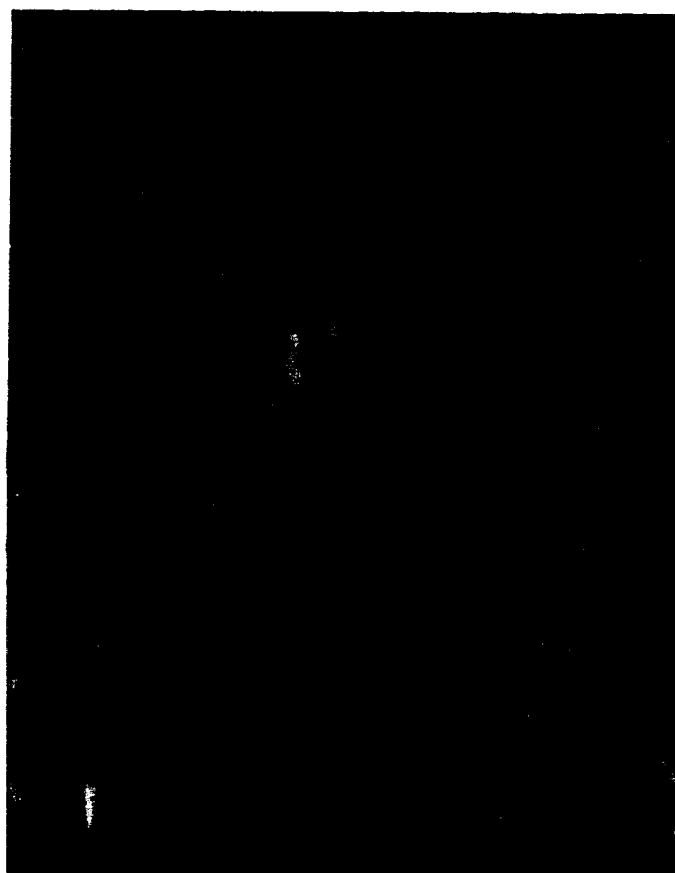


Figure 3(2)

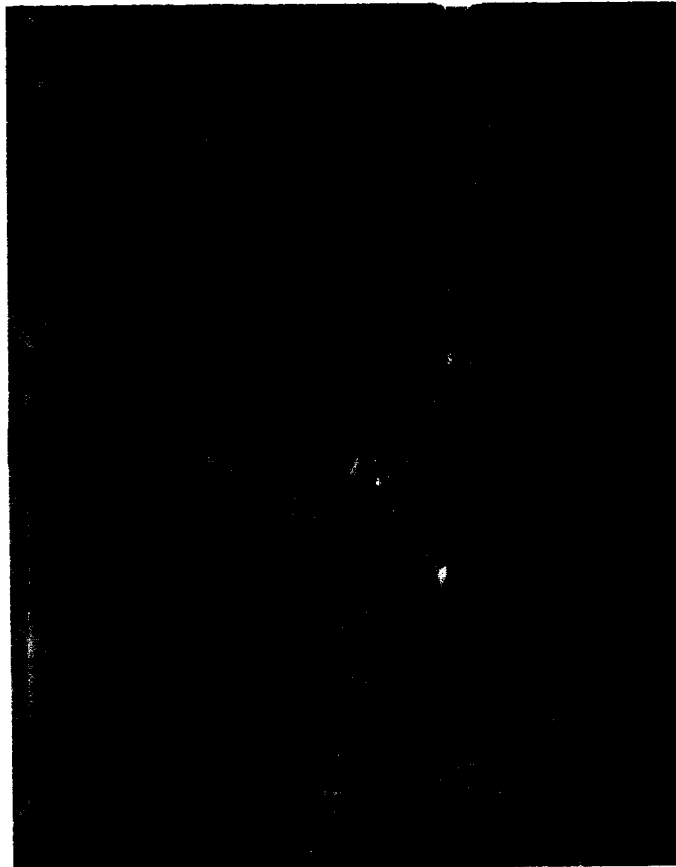


Figure 3(3)



Figure 4(1)

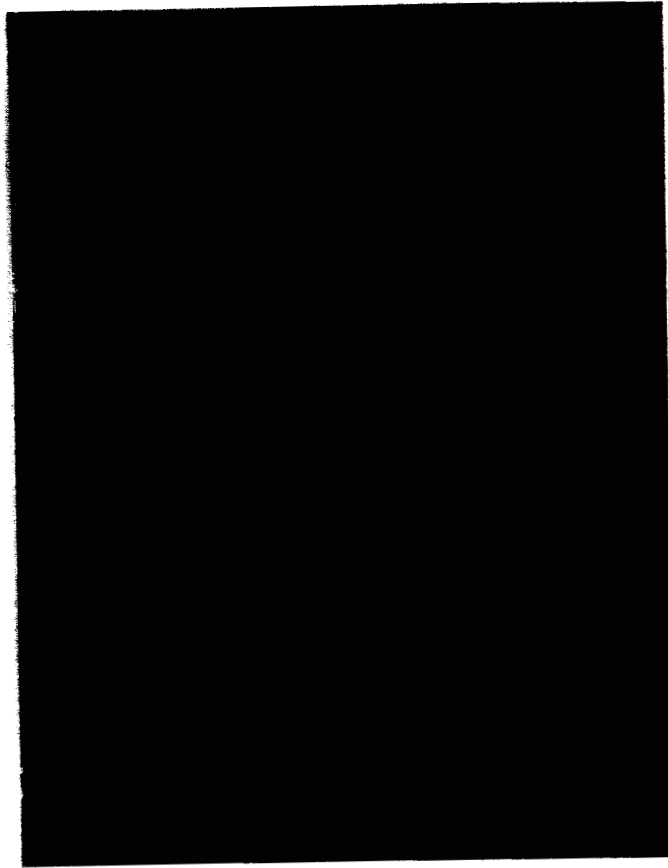


Figure 4(2)

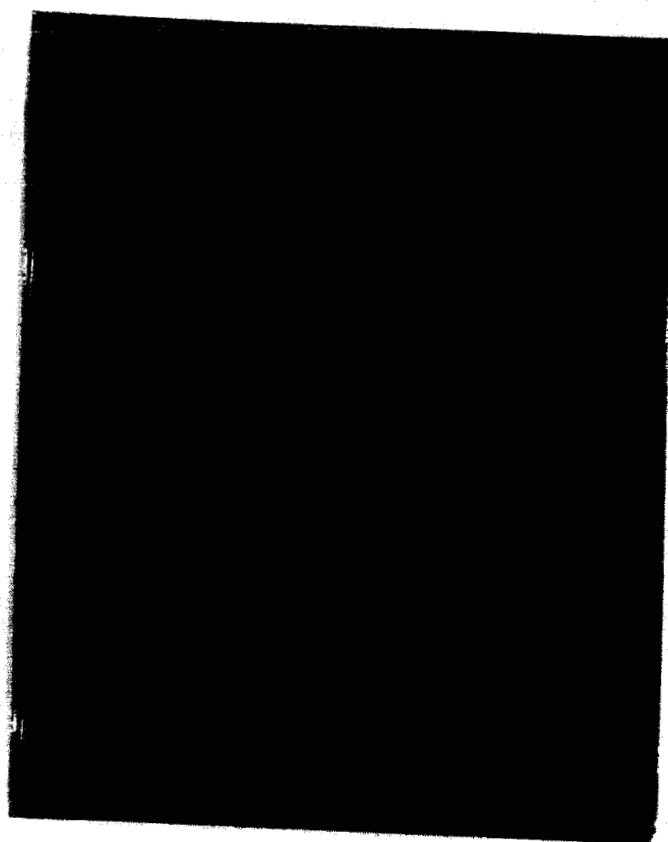


Figure 4(3)



Figure 5



Figure 6

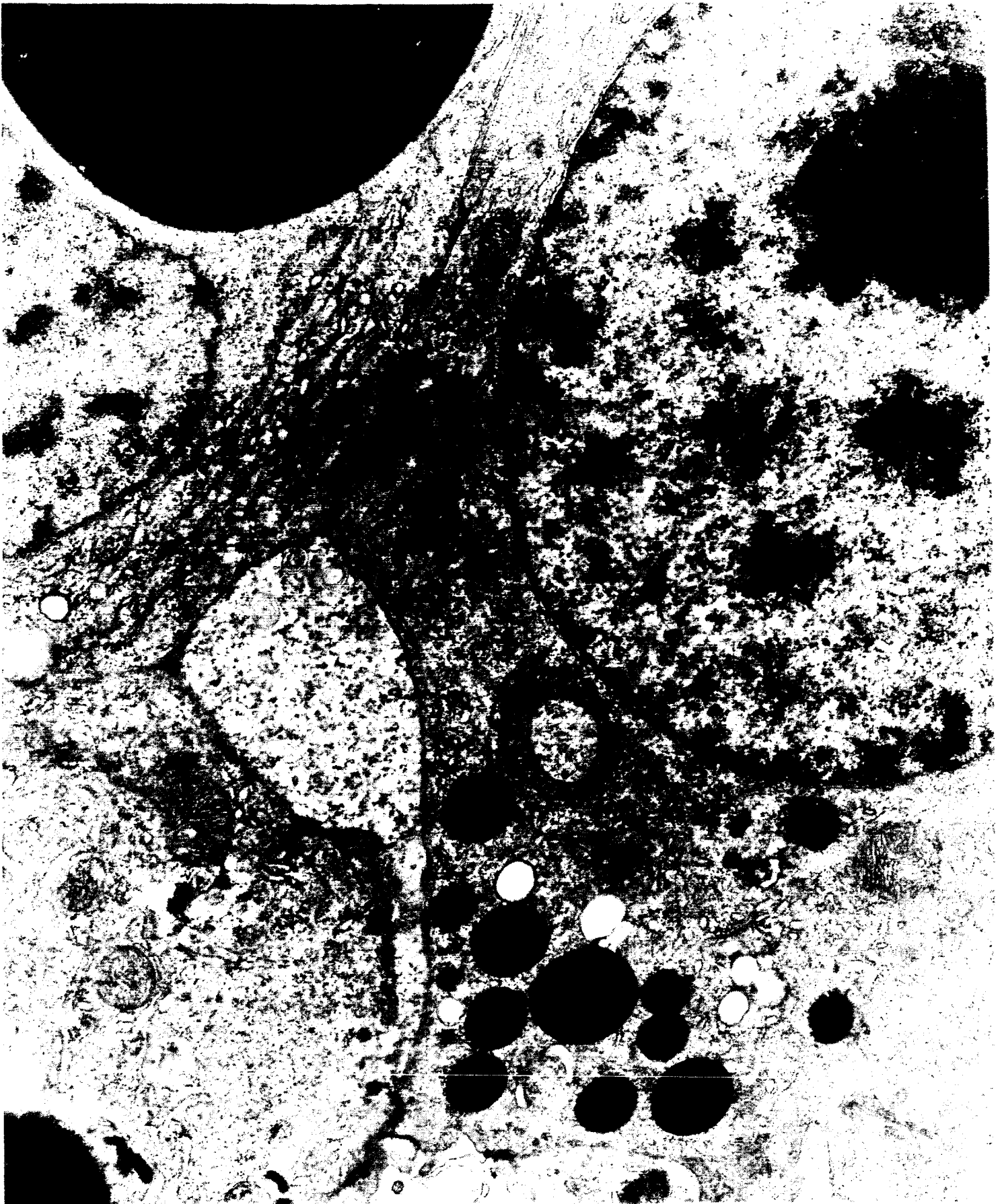


Figure 7



Figure 8



Figure 9